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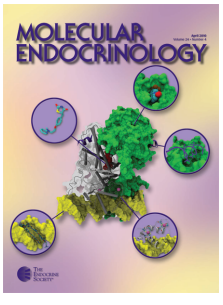

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Increased Levels and Pulsatility of Follicle-Stimulating Hormone in Mothers of Hereditary Dizygotic Twins*

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ABSTRACT

According to the endocrine model of hereditary dizygotic twinning, high FSH is responsible for multiple ovulation and pregnancy. Our study explored the underlying neuroendocrine causes.

In a prospective clinical study, we compared the third day of menses parameters of episodic secretion of LH and FSH, the pituitary response to LHRH, plasma estradiol, and dimeric inhibin A and B in 16 regularly menstruating and 9 postmenopausal mothers of dizygotic twins with a family history of twinning and 14 premenopausal and 9 postmenopausal controls. Seven of 16 premenopausal mothers of twins had abnormally high FSH levels of more than 10 IU/L compared with 1/14 in controls ($P = 0.024$). In the premenopausal mothers of twins, mean FSH concentrations ($P = 0.025$) and FSH pulse frequency ($P = 0.003$) were significantly elevated, whereas FSH pulse amplitude and FSH response to LHRH were unaltered. For LH, neither the secretory parameters nor the response to LHRH was different. There were no differences between estradiol and inhibin A and B levels. Postmenopausal mothers of twin and controls did not differ with respect to the secretory pattern of LH and FSH.

We conclude that under equal ovarian feedback conditions, premenopausal mothers of a dizygotic twin have hyper stimulation by endogenous FSH caused by neuroendocrine, hypothalamic, or pituitary mechanisms. This is the result of altered responsiveness to ovarian feedback and/or pituitary or suprapituitary, non-LHRH-like mechanisms that stimulate pulsatile FSH. (*J Clin Endocrinol Metab* **83:** 481–486, 1998)

NATURAL dizygotic twinning often occurs on a hereditary basis (1, 2). Furthermore, maternal age above 35 yr carries an increased risk of dizygotic twinning (3). Multiple follicle growth and subsequent multiple ovulation seem a logical explanation. Both have been demonstrated in mothers of hereditary dizygotic twins (4, 5). Usually, ongoing development of one dominant follicle takes place when a certain threshold in the level of plasma FSH is only marginally exceeded (6, 7). Multiple follicle growth is related to FSH levels higher than this threshold or to levels exceeding the threshold for too long a time (8). Milham (9) introduced an endocrinological model explaining the epidemiological observations for dizygotic twinning. According to this model, pituitary gonadotropins such as FSH and LH are responsible for the increased ovulation in mothers of dizygotic twins. Several studies have shown increased plasma gonadotropin levels in mothers of dizygotic twins (10–12).

Our study tested the hypothesis that a pituitary or suprapituitary hereditary difference exists that causes increased FSH levels in mothers of hereditary dizygotic twins. Both LH and FSH are clearly secreted in a pulsatile fashion during the early follicular phase (13). Pulsatile LH can be considered a good representation of the episodic activity of the hypothalamic LHRH pulse generator. Obviously, in cases of elevated FSH levels, detailed analysis of its episodic secretion, in concert with that of LH, could potentially reveal responsible mechanisms. We report here the pulsatile secretion of LH and FSH and the responsiveness to a test dose of LHRH in mothers of hereditary dizygotic twins compared with controls. Measurements took place on the third day of menstruation during the luteo-follicular transition of the cycle when recruitment of new follicles takes place (8). To evaluate the role of the ovary as a potential cause of differences in gonadotropin secretion, the levels of estradiol and dimeric inhibin A and B were also measured. Inhibin A, which is produced by the ovary in the luteal phase, and inhibin B, which is produced by the ovary in the follicular phase, are now clearly considered to be of physiological importance in the regulation of the menstrual cycle because of their selective negative feedback of FSH secretion (14). In a further attempt to define ovarian factors in hereditary dizygotic twinning, episodic gonadotropin secretion was evaluated in absence of ovarian feedback, *i.e.* after menopause, in mothers of twins and controls.

Subjects and Methods

Subjects

The study was approved by the local Committee of Ethics on Research in Human Subjects. Mothers of dizygotic twins were recruited by means of an advertisement in a annually published newsletter sent to all volunteers being registered in the Dutch Twin Register. This register is kept up to date at the division of Psychonomics from the Faculty of Psychology of the Free University of Amsterdam. Volunteering mothers of twins had to have given birth to spontaneous dizygotic twins of unequal

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sex before the age of 36 yr and had to have had at least one other dizygotic twin in a first or second degree female relative. They had to be in good general health without any current medical treatment. Women with hormonal treatment in the 6 months preceding the study were excluded. Premenopausal mothers had to have a regular menstrual cycle varying between 21–35 days in length. Postmenopausal mothers had to be at least 3 yr after menopause. The control singleton mothers had to meet the same criteria but with the requirement of having given birth to at least two singleton pregnancies before the age of 36 yr and had to be without twins in the family.

Blood sampling

Blood samples were collected on the third day of menstruation in the premenopausal group. All participants kept a basal body temperature chart to verify ovulation in the study cycle. In the postmenopausal group, the blood sampling took place on a convenient working day.

Serial blood samples for LH and FSH determination were taken every 10 min for 6 h via an indwelling catheter with a heparin lock in a convenient forearm vein. Sampling was always started between 0800–0900 h. Immediately after the last sample, an LHRH challenge with an iv injection of 100 μg LHRH (HRF, Wyeth, Hoofddorp, The Netherlands) followed, and three additional samples were taken after 30, 60, and 90 min for LH and FSH measurement.

Assays

Dimeric LH and FSH were measured in duplicate by means of a double first monoclonal and second polyclonal antibody immunoradiometric assay (IRMA-mat BYK-Sangtec Diagnostica GmbH & Co., Dietzenbach, Germany) with a lower detection limit of 0.2 IU/L. References for expression of LH and FSH were first International Reference Preparations 68/40 and second International Reference Preparations 78/549, respectively. LH intraassay coefficients of variation (CV) were 4.3% and 2.4% at the levels of 3 U/L and of 23 U/L, respectively. FSH intraassay CVs were 3% and 1.8% at levels of 7 U/L and 74 U/L, respectively. Interassay CVs were below 8% for both gonadotropins. For each individual, all samples were measured in the same assay for each hormone.

Inhibin A and B were measured in duplicate with two-site enzyme-linked immunoassays (Serotec, Oxford, UK) (14, 15). CVs were less than 5% within a plate, and less than 7% between plates of both assays. The sensitivity was 3 pg/mL for inhibin A and 15 pg/mL for inhibin B. Both hormones were measured in duplicate. Estradiol was measured by means of radio immunoassay (Sorin Biomedical, Silage, Italy) with a lower limit of detection of 18 pmol/L and CVs of less than 5%.

Pulse analysis

The analysis used was a computerized version of a previously developed and validated method (16, 17). The algorithm is valid for replicate repeated measurements of LH and FSH, with a less than 5% chance of indication of nonexisting pulses in series of 100 samples taken from pooled serum. This method is of particular value in detecting episodic secretion of hormones with relatively long half-lives, because pulses are indicated when a significant rise occurs without the requirement of a subsequent decline. Nadirs preceding the pulses are indicated as marker points in the hormone patterns rather than the pulses themselves.

Statistical analysis

For each subject, the mean concentrations of LH and FSH, the mean pulse amplitude over the day, and the frequency of LH and FSH pulses per 6 h were calculated. The maximal gonadotropin increment was taken as a parameter for the response to the LHRH challenge.

LH and FSH pulses were indicated to have occurred not synchronously when their beginnings were more than 10 min apart (18). From each subject, the number of FSH pulses that occurred singularly or in concordance with an LH pulse was estimated, and means per group were compared.

Hormone levels were transformed logarithmically before statistical analysis. Student's *t* test was used for comparison of means. Linear regression analysis was performed with the following dependent variables: mean concentrations, pulse frequencies per 6 h, pulse amplitudes, and responses to LHRH challenge. Twinning feature and smoking habits, expressed as number of pack years as a potential confounder, (19) were the independent variables. Fisher's exact test was used to evaluate significance of incidence of occurrence of high FSH values. Two- sided $P < 0.05$ was considered to indicate statistical significance.

Results

Basic characteristics of study population

We studied 16 premenopausal mothers of dizygotic twins and 14 controls. Their basic characteristics are summarized in Table 1. The mothers of twins did not differ from the controls with respect to age, parity, body mass index, and time from last delivery. However, they had smoked significantly more and their menstrual cycle length was 2 days less.

Nine postmenopausal mothers of twins and eight controls met the inclusion criteria. The two groups did not differ with respect to any of the basic characteristics (Table 2).

FSH secretion

The pulsatile nature of FSH and LH secretion was clearly discernible in both groups. Figure 1 shows the patterns of gonadotropin secretion in two premenopausal mothers of twins and two controls. Mean ± SD FSH levels (the overall average of all 37 samples per subject) of the premenopausal mothers of a dizygotic twin were 9.8 ± 5.5 IU/L, which is about 65% higher compared with control values of 6.0 ± 3.0 IU/L ($P = 0.025$). This coincided with a significant increase ($P = 0.003$) in FSH pulse frequency of 4.8 ± 1.5 pulses/6 h compared with 3.0 ± 1.5 pulses/6 h (Fig. 2). In mothers of twins, higher FSH levels were significantly associated with an increase in FSH pulse frequency (linear regression analysis: $P = 0.0215$). After correction for the differences in smok-

TABLE 1. Baseline characteristics (mean ± SD) of premenopausal mothers of dizygotic twins and controls

Characteristic	Controls (n = 14)	Twin mothers (n = 16)	*P*
Age (yr)	35.1 ± 3	35.9 ± 3.4	0.500
Body mass index (kg/m^2)	22.2 ± 3.9	23.0 ± 2.8	0.511
Cycle length (days)	28.4 ± 2.7	26.4 ± 2.2	0.037
Smoking (no. of pack years)	3.3 ± 5.6	7.0 ± 8.9	0.051
Days from last delivery	1857 ± 1300	1615 ± 1153	0.597
Parity	2.2 ± 0.4	1.9 ± 0.9	0.351
Age at twin delivery (yr)		30.0 ± 5.2	

TABLE 2. Baseline characteristics (mean ± SD) of postmenopausal mothers of dizygotic twins and controls

Characteristic	Controls (n = 8)	Twin mothers (n = 9)	*P*
Age (yr)	59.4 ± 9.0	55.3 ± 6.2	0.311
Body mass index (kg/m^2)	23.6 ± 2.6	25.2 ± 4.2	0.340
Smoking (no. of pack years)	4.1 ± 5.0	3.1 ± 4.8	0.704
Age at menopause	48.2 ± 3.6	47.2 ± 4.3	0.609
Years after menopause	11.1 ± 11.4	8.1 ± 6.9	0.530
Parity	3.1 ± 1.1	3.1 ± 1.3	0.981
Age at twin delivery (yr)		29.1 ± 3.6	

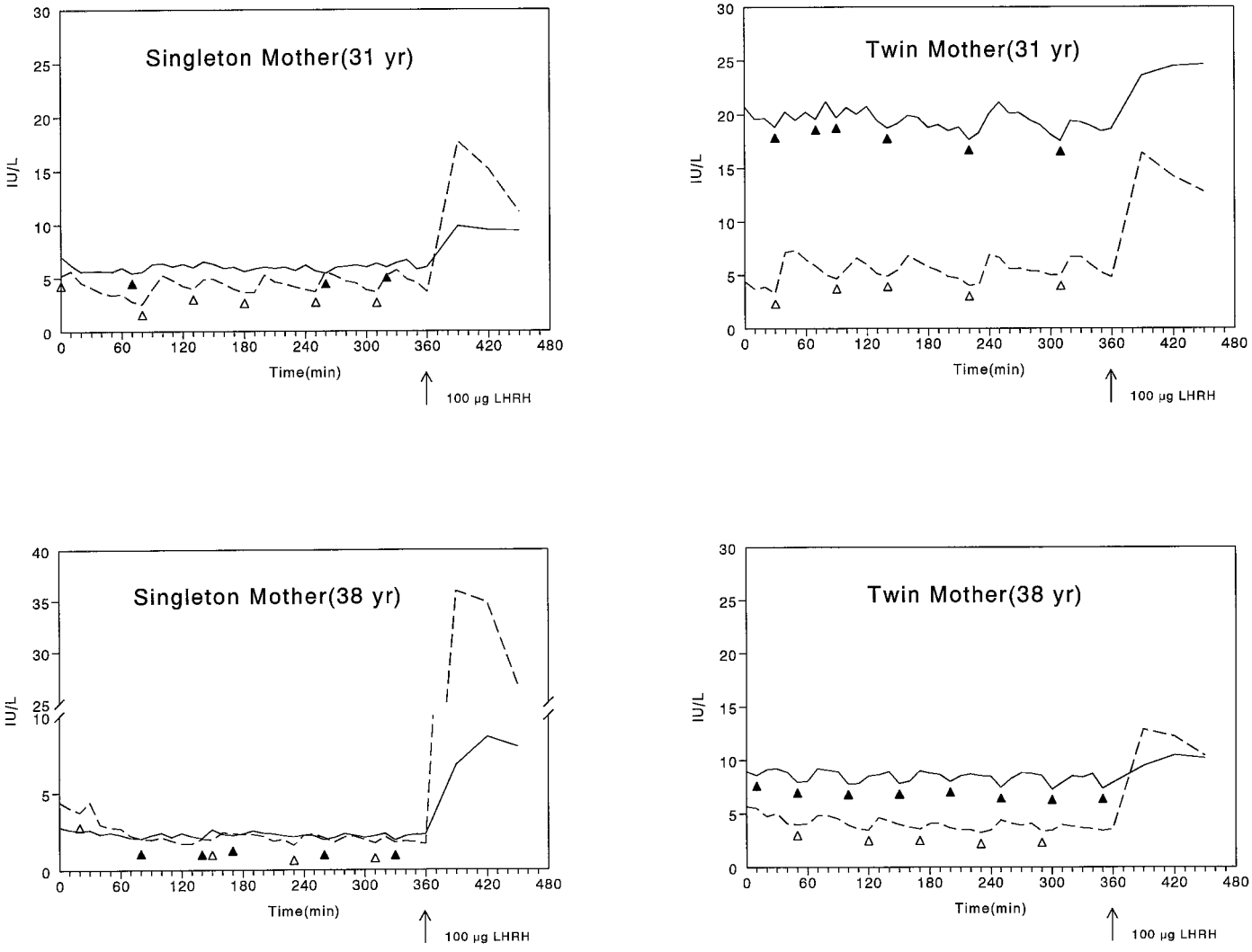

FIG. 1. Examples of secretion pattern and response to LHRH in two mothers of hereditary twins and two mothers of singletons. *Solid lines* represent FSH; *dashed lines* represent LH. ▲, Denote start of an FSH pulse; △, indicate LH pulses.

ing habits, the tendency for twinning still was shown to be an independent determinant for elevation of FSH levels and pulse frequency. In the linear regression model, age and body mass did not appear to be a determinant of FSH level. The mean FSH pulse amplitude and the maximum response to LHRH challenge were not significantly different. Compared with controls (1/14), significantly more mothers of twins (7/16) had one or more individual FSH values above 10 IU/L ($P = 0.024$). In the postmenopausal mothers of twins, FSH secretory parameters were not different from controls (Fig. 2).

LH secretion and ovarian feedback

In the premenopausal and postmenopausal mothers of twins, the parameters for episodic LH secretion did not differ from controls (Fig. 2). In the premenopausal mothers, mean plasma levels of 17-β estradiol and dimeric inhibin A and B levels showed no difference between the two groups (Table 3).

Copulsatility

The mean number and range of FSH pulses occurring without a concurrent LH pulse was a mean of 2.19 (range, 0–7) in mothers of twins, which was significantly more compared with controls (mean 0.93; range, 0–4; $P = 0.033$). The number of FSH pulses that had occurred synchronous with an LH pulse was not significantly different. In the postmenopausal mothers, no differences were observed with respect to number of individual LH and FSH pulses.

Discussion

In the present study, we attempted to show more precisely the site of origin of any difference in gonadotropin secretion related to dizygotic twinning. In line with Milham's (9) hypothesis and in agreement with earlier findings by Martin *et al.* (10, 11) we observed elevated follicular phase levels of FSH in mothers with a hereditary history of dizygotic twins. We found that this is associated with a rise in the FSH pulse

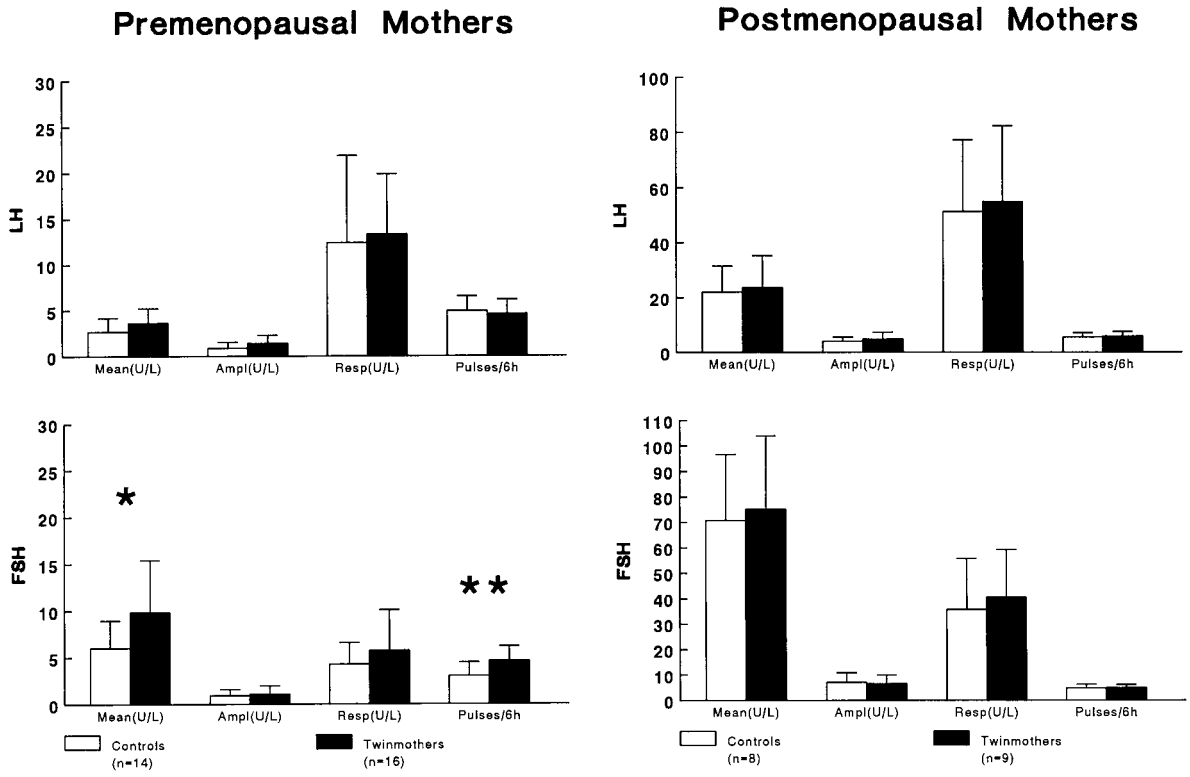

FIG. 2. Means and SD of different parameters of episodic LH (*upper panels*) and FSH (*lower panels*) in premenopausal (*left side*) and postmenopausal (*right side*) mothers of dizygotic twins (*solid bars*) and controls (*open bars*). *, $P = 0.025$; **, $P = 0.003$.

TABLE 3. Characteristic (mean ± SD) of ovarian feedback on cycle day 3 in premenopausal mothers of dizygotic twins (n = 16) and controls (n = 14)

Characteristic	Controls	Twin mothers	*P*
Estradiol (pmol/L)	140 ± 82	192 ± 170	0.350
Inhibin A (pg/mL)	5.4 ± 2.8	4.7 ± 2.3	0.413
Inhibin B (pg/mL)	77.8 ± 42.6	75.6 ± 46.0	0.901

frequency without any changes in other characteristics of pulsatile FSH.

Furthermore, we evaluated ovarian feedback by measuring estradiol levels and inhibin A and B to explain the elevated FSH levels. We found no changes in any of these negative feedback hormones, which is in line with earlier human studies (5, 10, 11). In addition, in the Booroola sheep model for dizygotic twinning, which exhibits an autosomal dominant gene (FecB) associated with elevated FSH levels and localized to a region homologous to the human chromosome 4, there is no evidence for differences in bioactive inhibin secretion (20). This argues against diminished negative ovarian feedback as the primary cause of elevated FSH levels in mothers of dizygotic twins.

Our observation of no differences in the secretion of FSH in postmenopausal mothers of twins compared with controls would nevertheless suggest a role for the ovary. It should be noted, however, that under postmenopausal conditions, subtle differences in FSH secretion may easily be obscured by the great hourly and interindividual variability in secretion of this hormone. Furthermore, it is possible that despite equal levels of hormones exerting negative feedback as measured with immunoassays, differences may exist in biological activity. However, the inhibin assays that were used only measured intact dimeric hormone, which correlates well with biological activity (14). We cannot rule out the possibility that still unknown ovarian hormones that regulate feedback of gonadotropin secretion are involved. Specific FSH stimulatory substances like activins, which are homodimers of the β-chains of inhibin but not their binding protein follistatin, may be involved in the natural rise of FSH in aging women (21). Others claim however, that activin is not involved in the gonadal regulation of the menstrual cycle (22). A logical explanation of our observations would be that differences in response to gonadal feedback signals are present. This would favor the pituitary and/or the hypothalamus as the primary sites involved in altered gonadotropin secretion in mothers of dizygotic twins.

A difference in FSH moiety that would result in increased serum half-life, thereby accounting for greater circulating concentrations in the mothers of twins, does not seem likely, because in our study the higher FSH levels can be attributed to a clear increase in the number of pulses. Several observations rule out an altered activity of the LHRH pulse generator as the cause of the increase in FSH pulse frequency. In the first place, the increase only concerned additional FSH pulses without a simultaneous LH pulse. In the second place, FSH pulse amplitudes were unaltered and there was no change in maximal response to LHRH. This makes it unlikely that an increased pituitary sensitivity to smaller LHRH pulses has caused the additional FSH pulses. Finally, and

probably most convincing, is our observation that none of the dynamic parameters of the LH secretion were different in the mothers of the twins.

For the initiation and maintenance of FSH secretion, pulsatile LHRH is an absolute prerequisite (23). There are indications that once FSH synthesis has started, spontaneous release by the pituitary may occur without LHRH stimulation. In particular, this part of FSH secretion is under the control of negative feedback through inhibin (24). In addition to this autonomous FSH secretion, pulsatile FSH secretion occurs in association with exogenous or endogenous LHRH pulses (25, 26), but the link between LHRH and FSH pulses is much less than between LHRH and LH pulses. The occurrence of single FSH pulses in earlier studies in postmenopausal women (18, 25, 27) without a concomitant LH pulse, indicates the presence of LHRH-independent factors governing episodic FSH secretion. This is also supported by the recent observations in sheep of clear FSH pulses in jugular vein blood without significant LHRH elevations in serially sampled blood from pituitary portal blood vessels (28). Altered activity of such factors are probably involved in the increased number of FSH pulses in mothers of twins. There are several possibilities for the nature of this(ese) factor(s). In the first place, spontaneous pituitary FSH secretion may occur in temporary bursts independent of any releasing factor. However, there are no data available that would support such a secretory mechanism. Alternatively, mothers of dizygotic twins may have genes that are activated that code for a still-putative separate FSH releasing hormone (29, 30), or they may carry mutations in the gene coding for LHRH, resulting in synthesis of an LHRH- like peptide preferably inducing secretion of FSH over LH (31).

In our view, it seems valid to assume that an hereditary trait of having dizygotic twins is the result of endogenous hyperstimulation by FSH in a significant proportion of mothers. A high proportion of mothers of twins in this study had one or more FSH values over 10 IU/L. Within the specifications of the assay that was used in this study, this observation is usually associated with incipient ovarian failure when observed in regularly menstruating women. This is thought to be caused by a diminished ovarian reserve (32). There are indications that being a mother of a dizygotic twin (either on an hereditary or nonhereditary basis) is a risk for earlier menopause (33). In the present study however, the postmenopausal mothers of hereditary dizygotic twins did not differ with controls in age at menopause. This might have been the result of undetectable results because of small sample size, but it may also indicate that incipient ovarian failure and hereditary dizygotic twinning are different entities. On the other hand, the natural rise of follicular phase FSH levels, as observed in general after 30 yr of age, may be the underlying cause of the risk of nonhereditary dizygotic twinning (3) with increased maternal age. Somewhat to our surprise, we could not show a relation between age and FSH. Probably a substantially larger number of subjects are needed to find these differences. Anyway, it should be noted that not all patients with high FSH levels in the early follicular phase should be considered to have premature incipient ovarian failure.

In conclusion, the endocrine hypothesis of an elevated FSH in hereditary dizygotic twinning seems valid. Our results favor pituitary- or LHRH-independent factors from higher regions of the brain leading to more FSH and hereby causing dizygotic twinning.

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